EXHIBIT H

Antibodies

A LABORATORY MANUAL

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PURIFYING ANTIBODIES

Purified antibodies are required for a number of techniques. Table 8.1 lists several techniques that rely on purified antibodies, at least in some steps. In many of the examples listed in this table, purified antibodies are labeled with an easily detected "tag" (p. 319), and these labeled antibodies are then used to determine the presence of an antigen or another antibody. When labeled anti-immunoglobulin antibodies (p. 622) are used to measure the presence of other antibodies, it is seldom worthwhile to prepare and label these reagents yourself. They can be purchased from a number of commercial sources, where they are prepared and tested in large, economic batches. However, when labeled antibodies will be used to detect an antigen directly, the primary antibody must be purified first. Direct labeling also allows two antibodies to be compared in the same assay by marking them with different tags. In other instances, purified antibodies are necessary for different applications. For example, purified antibodies may lower the background in some assays or purification may be the easiest method to concentrate antibody solutions.

There are a wide variety of methods used to purify antibodies. The correct choice of purification method will depend on a number of variables, including the use for which the antibodies are intended, the species in which it was raised, its class and subclass if it is a monoclonal antibody, and the source that will serve as the starting material for the purification. Table 8.2 summarizes the possible sources of antibodies for purification. Also included in this table are the possible sources of antibody contamination and the expected level of purity. Both of these factors may influence the choice of starting material.

TABLE 8.1
Techniques That Require Purified Antibodies

Technique	Antibody use	Antibody type	Best sources	Comments
Cell Staining	Direct localization	Anti-antigen	Polyclonal or monoclonal	Prepare yourself
	Indirect localization	Anti-antibody	Polyclonal	Available commercially
Immunoassays	Direct detection	Anti-antigen	Monoclonal, but polyclonal OK in some cases	Prepare yourself
	Indirect detection	Anti-antibody	Polyclonal	Available commercially
Immunoblots	Direct detection	Anti-antigen	Polyclonal or monoclonal	Prepare yourself
	Indirect detection	Anti-antibody	Polyclonal	Available commercially
Immunoaffinity	Purification	Anti-antigen	Monoclonal	Prepare yourself

Table 8.3 summarizes the commonly used methods for antibody purification and also lists the advantages and disadvantages of each method. As can be seen from this table, it is often necessary to combine several methods to achieve the desired purification. Finally, Tables 8.4 and 8.5 compare the expected results of the different purification methods when using either polyclonal (Table 8.4) or monoclonal (Table 8.5) antibody sources.

Although no single suggestion can fulfill all of the requirements for different purification needs, most workers have found that purification on protein A beads is the most useful technique. This technique has become the method of choice for antibodies with high affinities for protein A.

Conventional Methods

Antibodies from serum or ascites can be purified using conventional methods involving precipitation and column chromatography. When similar techniques are used on tissue culture supernatants, the degree of purity achieved will be lower because of the lower concentrations of the specific antibodies. For tissue culture supernatants, purification using protein A beads (p. 309) or anti-immunoglobulin antibody affinity columns (p. 316) is recommended.